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CUTTING EDGE

Cutting Edge: Competition for APC by CTLs of Different Specificities Is Not Functionally Important During Induction of Antiviral Responses[1]

Hans Christian Probst,[2] Tilman Dumrese,[2] and Maries F. van den Broek[3]

The hypothesis that T cell competition for access to APC influences priming of CTL responses is a controversial issue. A recent study using OVA as a model Ag supports this hypothesis and received considerable attention. However, using a comparable approach, we reached a different conclusion. We analyzed whether TCR transgenic T cells specific for lymphocytic choriomeningitis virus gp33–41/D^b could inhibit the priming of endogenous responses against gp33–41 and against two other lymphocytic choriomeningitis virus glycoprotein-derived CTL epitopes. After priming with different stimuli, gp33–41/D^b-specific TCR transgenic T cells reduced the endogenous gp33–41/D^b response in a dose-dependent way, but all other endogenous responses were unaffected. Even when $>10^6$ TCR transgenic cells were combined with weak priming, no reduction of responses other than of those specific for gp33–41/D^b was observed. Thus, competition for APC by CTLs of different specificities is not of functional relevance in antiviral immune responses. ***The Journal of Immunology,*** **2002, 168: 5387–5391.**

In the control of infections with viruses or intracellular bacteria, $CD8^+$ T cells have been shown to be of crucial importance (1). $CD8^+$ T cells recognize 8- to 10-aa long peptide fragments presented on the cell surface by MHC class I molecules (2). Only a few of the numerous peptides encoded by pathogens are able to activate specific $CD8^+$ T cells, and responses to these epitopes are often dominated by one or two epitopes. This phenomenon is termed immunodominance (3–5). Knowledge of mechanisms that influence immunodominance is important to the understanding of $CD8^+$ T cell priming, viral evasion mechanisms, or the design of vaccines. Several factors that contribute to immunodominance have been identified, and their relative importance seems to depend on the experimental system used. These include the efficiency of epitope generation by the Ag-processing machinery, the affinity of the peptide for the presenting MHC class I molecule, and the abundance of T cells responding to this particular peptide-MHC class I complex (3, 5–11). Antigenic competition, in which immune responses to one determinant are inhibited by simultaneous exposure to (other) Ags on the same APC (immunodomination), has recently been suggested to contribute to immunodominance (5, 12–14). The observation that, upon secondary challenge (15–17) or in the memory phase (18) of an immune response, the affinity of the responding T cells increases without evidence for affinity maturation of the TCR is also interpreted in terms of T cell competition. Thus, T cells with a higher affinity must have an advantage in secondary responses (where usually the antigenic load is low), suggesting that T cells compete for Ag. Obviously, in the aforementioned experiments (15–18), competition is studied between T cells with the same specificities but different TCR affinities.

Whether T cells with different specificities compete with each other for access to APC has been addressed in three systems with studies using: 1) C57BL/6-anti-BALB.B minor histocompatibility Ags (12, 13); 2) H-Y and $B6^{dom1}$ minor histocompatibility Ags (19, 20); and 3) OT-1 transgenic T cells (specific for the OVA-derived peptide SIINFEKL (S8L)/K^b) combined with infection with recombinant vaccinia virus (VV)[4]-expressing OVA or priming by dendritic cells (DC) loaded with antigenic peptides (14). In the studies using transplantation Ags, elimination of APC by dominant CTL (faster, more) was suggested as a mechanism, whereas the study using OVA claimed that T cell competition for access to APC was responsible.

To study whether T cell competition for Ag-bearing APC is a mechanism of general importance in immunodominance, we adoptively transferred titrated numbers of TCR transgenic $CD8^+$ cells (318, specific for lymphocytic choriomeningitis virus (LCMV) glycoprotein-derived 33–41/D^b) together with priming for LCMV-derived CTL epitopes. LCMV is a potent system for studying immunodominance, as a strong and well-characterized CTL response directed against three LCMV glycoprotein-derived immunodominant epitopes (gp33–41/D^b, gp34–41/K^b, gp276–286/D^b) is elicited. To prime different numbers of specific CTL, mice were injected with LCMV, gp1–60 transgenic DC, or VV-G2 (expressing LCMV glycoprotein). Analysis of the endogenous CTL response against all immunodominant epitopes showed that no inhibition of

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[4] Abbreviations used in this paper: VV, vaccinia virus; DC, dendritic cell; LCMV, lymphocytic choriomeningitis virus; p.i., postinfection.

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endogenous CTL response against epitopes other than gp33 occurred.

Materials and Methods

Mice

C57BL/6 mice were obtained from the Institut für Labortierkunde (University of Zürich, Zürich, Switzerland). B6.PL (Thy1.1) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Transgenic mice expressing the LCMV glycoprotein aa 1–60 (H8 mice) have been described previously (21), as have the mice expressing the P14 TCR recognizing LCMV gp33–41/H-2D^b (318 mice; Ref. 22). For adoptive transfer experiments, 318 × B6.PL (318.PL) mice were used as donors. All mice were on a C57BL/6 background. Male mice of 6–10 wk of age were used.

Viruses

LCMV-WE was originally obtained from Dr. F. Lehmann-Grube (University of Hamburg, Hamburg, Germany) (23) and was propagated on L929 cells at a low multiplicity of infection. rVV encoding the LCMV glycoprotein (VV-G2) was obtained from Dr. D. Bishop (Institute of Virology, Oxford, U.K.) and was propagated on BSC40 cells (24).

Generation of bone marrow-derived DC

Bone marrow-derived DC were generated from femora of H8 mice as previously described (25). DC were cultured in the presence of the agonistic anti-CD40 mAb FGK45 (50 μg/ml; Ref. 26) during the last 48 h.

Adoptive transfer and infection

318.PL CD8$^+$ splenocytes were positively selected on a VS/LS column (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The selected population contained >95% CD8$^+$Thy1.1$^+$ cells. CD8$^+$ cells were injected into C57BL/6 mice. After CD8$^+$ transfer, mice were infected with 10^2 PFU LCMV-WE or with 10^6 PFU VV-G2. Alternatively, mice were injected immediately after adoptive transfer with H8 DC. Virus, CD8$^+$ cells, and DC were injected in 0.2 ml balanced salt solution into the tail vein.

Abs, tetramers, and flow cytometry

MHC class I tetramers were produced as previously described (27). PE-labeled anti-CD90.1 (Thy1.1) (clone HIS51) and FITC-labeled anti-CD8α (clone 53-6.7) were obtained from BD PharMingen (San Diego, CA).

At indicated timepoints, blood samples were stained for 10 min at 37°C with tetramer, followed by staining with anti-CD8FITC + anti-Thy1.1PE for 30 min at 4°C. Samples were washed twice, erythrocytes were lysed with FACS lysis solution (BD Biosciences, Mountain View, CA), and analyzed on a FACScan using CellQuest software (BD Biosciences).

Determining viral titers

LCMV titers were measured in the spleens of infected mice at the indicated timepoints. Organs were homogenized and monolayers of MC57G cells were infected with 10-fold dilutions of the homogenate for 48 h. LCMV was detected by intracellular staining with a monoclonal rat anti-LCMV nucleoprotein (VL4) as described (28). VV titers were determined in ovaries: confluent monolayers of BSC40 cells were infected with 10-fold dilutions of the ovary homogenates. Plaques were visualized by crystal violet after 48 h.

Results

Transferred LCMV gp33-41/D^b-specific TCR transgenic (318) CD8$^+$ cells inhibit the endogenous gp33-41/D^b response, but leave other endogenous, LCMV-specific responses intact

Infection of C57BL/6 mice with 100 PFU LCMV-WE primed substantial numbers of CD8$^+$ cells for each of the three LCMV gp-derived immunodominant epitopes. Mice were bled 12 days after infection (peak of the response in the blood) and cells were stained with Cychrome-labeled tetramers, PE-labeled anti-Thy1.1 and FITC-labeled anti-CD8 Abs. The percentage of endogenous specific CTL was determined within the CD8$^+$Thy1.1$^-$ population.

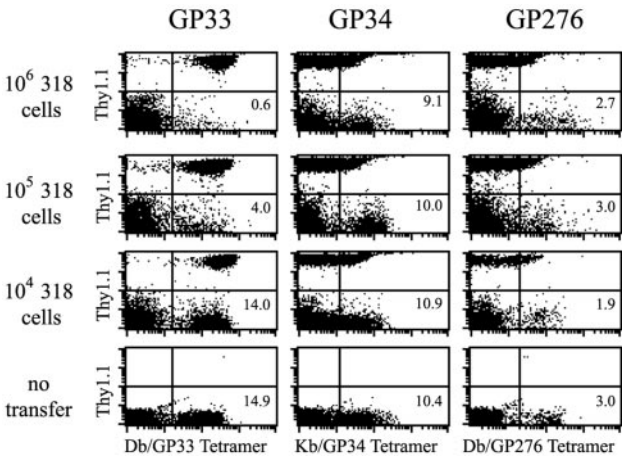

FIGURE 1. Transferred LCMV gp33-41/D^b-specific TCR transgenic (318) CD8$^+$ cells compete against the endogenous gp33-41/D^b response, but not against other endogenous LCMV-specific responses. C57BL/6 mice were adoptively transferred with titrated numbers of 318.PL CD8$^+$ cells and were infected with 100 PFU LCMV-WE. Twelve days after infection, PBL were stained with Cychrome-labeled gp33-41D^b, gp34-41K^b, or gp276-286D^b tetramers, with anti-CD8-FITC and with anti-Thy1.1-PE as described in *Materials and Methods*. Data were analyzed by gating on CD8$^+$ cells; Thy1.1$^+$ cells represent the adoptively transferred 318 cells, whereas Thy1.1$^-$ cells represent the endogenous response. The number given in the lower right quadrant represents the percentage of tetramer-positive cells of the endogenous response. Representative stainings are shown.

We found that 14.9% of CD8$^+$ cells were specific for gp33, 10.4% for 34, and 3.0% for gp276 (Fig. 1). Adoptive transfer of titrated numbers of naive MACS-purified CD8$^+$ cells from 318.PL mice that carry a transgenic TCR specific for LCMV gp33 in ~50% of their CD8$^+$ cells, together with infection with 100 PFU LCMV-WE, reduced priming of the endogenous (Thy1.1$^-$) CD8$^+$ cells for gp33 in a dose-dependent way (Fig. 1), demonstrating that competition between T cells of the same specificity occurred. The total number of gp33 CTL (endogenous + transferred) in the spleen 8 days postinfection (p.i.) varied between 3.4–8.9 × 10^6 gp33 CTL, and was relatively independent of the number of adoptively transferred 318 cells (not shown). This shows that increasing the CTL precursors does not necessarily increase the response (29, 30). The competition we observed is well in line with the experiments published by Kedl et al. (14) who found that adoptive transfer of TCR transgenic, SIINFEKL/K^b-specific OT-1 cells inhibited the priming of SIINFEKL/K^b-specific CD8$^+$ cells by VV-OVA. However, the endogenous response to the other two LCMV glycoprotein-derived immunodominant epitopes was not affected by adoptive transfer of 318.PL cells: even after transfer of as many as 10^6 TCR transgenic CD8$^+$ cells, there was no difference to nontransferred LCMV-WE-infected mice with respect to the percentage of CD8$^+$Thy1.1$^-$ cells specific for gp34 (9.1%) or for gp276 (2.7%), whereas the endogenous gp33 response was drastically reduced to 0.6% (Fig. 1). We found similar results at days 8 and 30, and also when we compared blood and spleen (data not shown). In addition, similar results were obtained after infection of mice with 10^6 PFU LCMV-WE (data not shown). Transfer of 318 cells reduced LCMV loads from days 4 to 5 on; titers in the spleen were similar in mice that received no cells or 3 × 10^4 or 10^6 TCR transgenic cells on days 2 and 3 p.i. On day 4 p.i., 3 × 10^4 318 cells did not reduce the titer, but 10^6 318 cells reduced the titer 2-fold. On day

5 p.i., 3×10^4 318 cells reduced titers 2-fold, and 10^6 318 cells reduced titers 100-fold.

Adoptively transferred LCMV gp33–41/D^b-specific TCR transgenic CD8$^+$ cells do not inhibit priming of endogenous CD8$^+$ responses to other LCMV-derived epitopes, not even when priming is weak

In the experiments described above, we found no evidence for T cells that inhibit priming of T cells specific for other epitopes presented by the same APC. Because infection with LCMV, a noncytopathic virus that is known to replicate well in immunocompetent mice, primes a vehement CTL response, we reasoned that priming with a weaker infectious agent that putatively gives rise to less LCMV-presenting APC might reveal evidence for T cell competition for access to Ag-bearing APC. Therefore, we transferred 318 CD8$^+$ cells into C57BL/6 mice infected with 100 PFU LCMV-WE or with 10^6 PFU VV-G2, and measured the endogenous CTL response against all immunodominant LCMV glycoprotein-derived CTL epitopes expressed by both pathogens 9 days later (peak response in the blood after VV priming). Infection with LCMV-WE confirmed the data shown in Fig. 1. As little as 10^4 318 CD8$^+$ cells (equivalent to 5000 gp33-specific cells) already inhibited the priming of endogenous gp33-specific response to some extent; considerable (LCMV-WE) to almost complete (VV-G2) inhibition was observed after transfer of 10^5 318 CD8$^+$ cells. No inhibitory effect was seen on the priming of the two other immunodominant specificities, not even after transfer of 10^6 318 CD8$^+$ cells (Fig. 2*A*). Even after very weak priming with VV-G2 (compare the percentages of tetramer-positive cells of Fig. 2*B* with those of Figs. 2*A*), we found no evidence for inhibition of priming of the endogenous gp34 or gp276 response, whereas priming of the endogenous gp33 response was clearly inhibited (Fig. 2*B*). We found no evidence for enhanced VV elimination due to transferred 318 cells (up to 10^6) as measured in the ovaries 5 days after infection. Importantly, tetramer-positive cells of all three specificities were fully functional independent of the number of transferred 318.PL CD8$^+$ cells as determined by chromium-release assay (not shown).

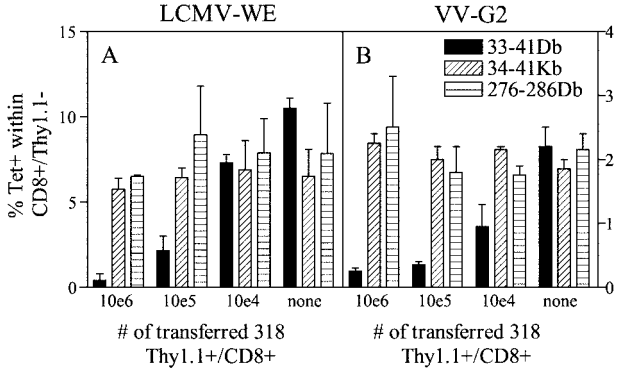

FIGURE 2. Transferred LCMV gp33-41/D^b-specific TCR transgenic (318) CD8$^+$ cells compete against the endogenous gp33-41/D^b response, but not against other endogenous LCMV-specific responses, and this is independent of antigenic load or the degree of priming. Titrated numbers of 318.PL CD8$^+$ cells were adoptively transferred into C57BL/6 mice, followed by infection with (*A*) 100 PFU LCMV-WE, or (*B*) 10^6 PFU VV-G2 (expressing LCMV glycoprotein). FACS staining was done as described in Fig. 1. Data represent the mean ± SD of three individual mice bled at day 9 after infection. This experiment was performed three times with similar results.

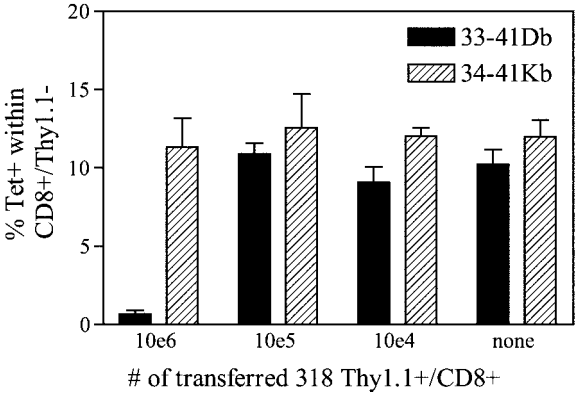

FIGURE 3. Transferred LCMV gp33-41/D^b-specific TCR transgenic (318) CD8$^+$ cells compete against the endogenous gp33-41/D^b response, but not against the gp34-41/K^b response after priming with 10^5 gp1-60 expressing DC (H8 DC). Titrated numbers of 318.PL CD8$^+$ cells were adoptively transferred together with H8 DC into C57BL/6 recipients. FACS staining was done as described in Fig. 1. Data represent the mean ± SD of three individual mice bled at day 7 after priming. This experiment was performed two times with similar results.

Transferred LCMV gp33-41/D^b-specific TCR transgenic (318) CD8$^+$ cells compete against the endogenous gp33-41/D^b response, but not against the gp34-41/Kb response after priming with gp1-60 expressing DC (H8 DC)

We primed C57BL/6 mice with titrated numbers of transgenic H8 DC (23) combined with transfer of titrated numbers of 318.PL CD8$^+$ cells. H8 DC continuously present both gp33 and gp34 and have the advantage over peptide-loaded DC that off-rates of peptides are not confusing the experimental system. Mice were bled at day 7, at which the peak response after DC priming is seen. We found that mice injected with 10^5 H8 DC, together with titrated numbers of TCR transgenic CD8$^+$ cells, displayed a dose-dependent inhibition of priming of endogenous gp33 CTL, but did not affect priming of gp34 CTL (Fig. 3). Similar results were obtained if mice were primed with 10^6 H8 DC (not shown).

Discussion

T cell competition for Ag-expressing APCs has been suggested as a mechanism that can determine the selective outgrowth of higher affinity T cells (15–18), but that can also play an important role in immunodominance. The experiments described in this study were designed to address the controversial issue of T cell competition for APC.

The feature of T cell competition has been addressed before, using different setups. The experimental system used by Perreault and colleagues (19, 20) is based on adoptively transferring male C57BL/6 (H-Y and B6^{dom1}), female C57BL/6 (B6^{dom1}), or male C3H.SW (H-Y) cells into female C3H.SW mice, followed by analysis of H-Y and B6^{dom1}-specific CTL. B6^{dom1}-specific CTL were found to inhibit priming of H-Y-specific CTL when both epitopes were presented by the same APC. B6^{dom1}-specific CTL were shown to expand more rapidly and eliminate APC before H-Y specific CTL were primed, resulting in immunodominance of B6^{dom1}. B6^{dom1}-specific CTL may expand faster, because of more efficient priming; as was shown in the same study, 800 B6^{dom1}/D^b, and only 8 H-Y/D^b, complexes were present on APC. The same mechanism was suggested to be operative in immunodominance in the C57BL/6-anti-BALB.B CTL response by Wolpert and colleagues (12, 13); they reported that CTL response specific for the

immunodominant epitope (H-28^c) developed faster than those specific for the subdominant epitopes (H-8^c, H-19^c, and H-25^c).

To explain T cell competition, a second hypothesis has been suggested by Kedl et al. (14). They suggested that "crowding" of T cells of one specificity on the APC physically inhibited the access of other T cells to this APC and, in addition, they excluded elimination of APC. This hypothesis received considerable attention recently. Kedl et al. (14) transferred titrated numbers of TCR transgenic OT-1 cells (specific for OVA-derived S8L) together with infection with VV-OVA, and analyzed priming of endogenous S8L- and KVVRVDKL (K8L) (a subdominant, K^b-restricted OVA-derived epitope)-specific CTL. They found that OT-1 cells inhibited the priming of both endogenous responses. In addition, they primed mice with peptide-loaded DC (S8L and SIYRYYGL, the K^b-restricted 2C epitope) together with OT-1 transfer and found that priming of endogenous SIYRYYGL-specific CTL was inhibited if S8L was present on the same DC, but not if the two peptides were on different DC. They interpreted these data such that large numbers of OT-1 cells interacted with the S8LS-presenting APC, thus inhibiting priming of other CTL. As CTL precursors usually make up a rather small population in a naive mouse that is dispersed over several lymphoid organs, it is difficult to conceive how this crowding should function under physiological conditions. Therefore, we used a comparable setup to further investigate the relevance of T cell competition for access to Ag-expressing APC. We adoptively transferred TCR transgenic 318 cells, together with priming with LCMV, VV-G2, or transgenic H8 DC, and analyzed the endogenous gp33 CTL response, as well as the response to two additional LCMV glycoprotein-derived epitopes (gp34 and gp276). We clearly could inhibit the endogenous gp33 CTL response by transfer of as few as 10^4 318 cells and completely inhibited the endogenous response by 10^5 or more 318 cells. This is in agreement with data published by others (14, 31), and illustrates that there is apparently a limit to the number of specific T cells that can be primed or that can expand in a host (29, 30, 31). This might be an important mechanism to disconnect precursor frequency and size of the immune response, thus allowing a response that is proportional to the antigenic load, as has been suggested before (29, 31). However, we could not reproduce the finding that T cells of one specificity inhibited priming of T cells of other specificities if these Ags were presented by the same APC; although the different ways of priming we used led to responses of different magnitude (between 1.5 and 10% tetramer-positive cells within the CD8 population), we never found inhibition of priming of the gp34 or of the gp276 response. Thus, in our experiments that were very similar to those published by Kedl et al. (14), we found no evidence for the hypothesis that CTL of one specificity can inhibit priming of CTL for another specificity by hampering access of the latter to the APC.

A possible explanation for this discrepancy might be that the OT-1 epitope (S8L) is presumably, due to its higher affinity for K^b, present in higher numbers on VV-OVA-infected APC than the subdominant epitope (K8L), resulting in more efficient (more and/or faster) priming of S8L-specific CTL, which was actually observed (0.4% S8L- and 0.07% K8L-specific CTL were primed by VV-OVA (14). As a comparison, VV-G2 primes around 2% for all three glycoprotein-derived epitopes). Thus, before K8L-specific CTL were able to interact with sufficient Ag to be substantially primed, the APC might have been eliminated. Although no effect of transferred OT-1 cells on VV-OVA clearance was reported (14), subtle effects on the number of APC, that are not reflected by reduction of virus titer may be sufficient to reduce the priming of the subdominant epitope. The discrepancy between the experiments using priming with DC might be explained by the fact that Kedl et al. used peptide-loaded DC, whereas we used DC that produce the Ags endogenously. In our study, off-rates from the presenting MHC class I molecules do not confuse the system, and in addition, may be more physiological, as infected APC will also continuously present Ags.

Thus, our data do not support the hypothesis that T cell competition for access to Ag-expressing APC due to crowding (14) is a phenomenon of functional importance in priming of antiviral immunity or in immunodominance. However, in some situations T cell competition has been shown to play a decisive role in immunodominance; in these studies, the mechanism has been shown to depend on differences in CTL priming and/or expansion as the major feature, resulting in elimination of APC by immunodominant CTL before the subdominant CTL could be substantially primed (12, 13, 19, 20).

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References

1. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. *Adv. Immunol. 27:52.*
2. Rammensee, H. G., K. Falk, and O. Rötzschke. 1993. Peptides naturally presented by MHC class I molecules. *Annu. Rev. Immunol. 11:213.*
3. Yewdell, J., and J. R. Bennink. 1999. Immunodominance in MHC class I restricted responses. *Annu. Rev. Immunol. 17:51.*
4. Sercarz, E., P. V. Lehmann, A. Ametani, G. Benichou, A. Miller, and K. Moudgil. 1993. Dominance and crypticity of T cell antigenic determinants. *Annu. Rev. Immunol. 11:729.*
5. Chen, W., L. C. Anton, J. R. Bennink, and J. W. Yewdell. 2000. Dissecting the multifactorial causes of immunodominance in MHC class I restricted T cell responses to viruses. *Immunity 12:83.*
6. Sette, A., A. Vitiello, B. Reherman, P. Fowler, R. Nayersina, W. Kast, C. Melief, C. Oseroff, L. Yuan, and J. Ruppert. 1996. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. *J. Immunol. 153:5586.*
7. Eisenlohr, L., J. Yewdell, and J. Bennink. 1992. Flanking sequences influence the presentation of an endogenously synthesized peptide to cytotoxic T lymphocytes. *J. Exp. Med. 175:481.*
8. Chen, W., S. Khilko, J. Pecondo, D. Margulies, and J. McCluskey. 1994. Determinant selection of MHC class I restricted antigenic peptides is explained by class I-peptide affinity and is strongly influenced by nondominant anchor residues. *J. Exp. Med. 180:1471.*
9. Restifo, N., I. Bacik, K. Irvine, J. Yewdell, B. McCabe, R. Anderson, L. Eisenlohr, S. Rosenberg, and J. Bennink. 1995. Antigen processing in vivo and the elicitation of primary CTL responses. *J. Immunol. 154:4414.*
10. Ossendorp, F., M. Eggers, A. Neisig, T. Ruppert, M. Groettrup, A. Sijts, E. Mengedé, P. Kloetzel, J. Neefjes, U. Koszinowski, and C. Melief. 1996. A single amino acid residue exchange within a viral epitope alters proteasome-mediated degradation resulting in lack of CTL priming. *Immunity 5:115.*
11. Deng, Y., J. Yewdell, L. Eisenlohr, and J. Bennink. 1997. MHC affinity, peptide liberation, T-cell repertoire, and immunodominace all contribute to the paucity of MHC class I restricted peptides recognized by antiviral CTL. *J. Immunol. 158: 1507.*
12. Wolpert, E., P. Grufman, J. Sandberg, A. Tegnejö, and K. Kärre. 1998. Immunodominace in the CTL response against minor histocompatibility complex antigens: interference between responding T cells, rather that with presentation of epitopes. *J. Immunol. 161:4499.*
13. Grufman, P., E. Wolpert, J. Sandberg, and K. Kärre. 1999. T cell competition for the APC as a model for immunodominance in the CTL response against minor histocompatibility antigens. *Eur. J. Immunol. 29:2197.*
14. Kedl, R., W. Rees, D. Hildeman, B. Schaefer, T. Mitchell, J. Kappler, and P. Marrack. 2000. T cells compete for access to antigen-bearing antigen-presenting cells. *J. Exp. Med. 192:1105.*
15. Busch, D., and E. Pamer. 1999. T cell affinity maturation by selective expansion during infection. *J. Exp. Med. 189:701.*
16. Savage, P., J. Boniface, and M. Davis. 1999. A kinetic basis for T cell receptor repertoire selection during an immune response. *Immunity 10:485.*
17. Rees, W., J. Bender, T. Teague, R. Kedl, F. Crawford, P. Marrack, and J. Kappler. 1999. *Proc. Natl. Acad. Sci. USA 96:9781.*
18. Slifka, M., and J. L. Whitton. 2001. Functional avidity maturation of CD8$^+$ T cells without selection of higher affinity TCR. *Nat. Immunol. 2:711.*
19. Pion, S., G. Christianson, P. Fontaine, D. Roopenian, and C. Perreault. 1999. Shaping the repertoire of CTL responses: explanation for the immunodominance

effect whereby CTL specific for immunodominant antigens prevent recognition of nondominant antigens. *Blood 93:952.*
20. Loyer, V., P. Fontaine, S. Pion, F. Hétu, D. Roy, and C. Perreault. 1999. The in vivo fate of APCs displaying minor H antigens and/or MHC differences is regulated by CTL specific for immunodominant class II-associated epitopes. *J. Immunol. 163:6462.*
21. Ehl, S., J. Hombach, P. Aichele, T. Rülicke, B. Odermatt, H. Hengartner, and R. M. Zinkernagel. 1998. Viral and bacterial infections interfere with peripheral tolerance induction and activate $CD8^+$ T cells to cause immunopathology. *J. Exp. Med. 187:763.*
22. Pircher, H., D. Moskophidis, U. Rohrer, K. Bürki, H. Hengartner, and R. M. Zinkernagel. 1990. Viral escape by selection of cytotoxic T cell resistant virus variants in vivo. *Nature 346:629.*
23. Lehmann-Grube, F. 1971. Lymphocytic choriomeningitis virus. *Virol. Monogr. 10:1.*
24. Hany, M., S. Oehen, M. Schulz, H. Hengartner, M. Mackett, D. Bishop, H. Overton, and R. M. Zinkernagel. 1989. Anti-viral protection and prevention of lymphocytic choriomeningitis or of the local footpad swelling reaction in mice by immunization with vaccinia-recombinant virus expressing LCMV-WE nucleoprotein or glycoprotein. *Eur. J. Immunol. 19:417.*
25. Ludewig, B., S. Ehl, U. Karrer, B. Odermatt, H. Hengartner, and R. M. Zinkernagel. 1998. Dendritic cells efficiently induce protective antiviral immunity. *J. Virol. 72:3812.*
26. Schoenberger, S., R. Toes, E. van der Voort, R. Offringa, and C. Melief. 1998. T cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature 393:480.*
27. Gallimore, A., A. Glithero, A. Godkin, A. Tissot, A. Plückthun, T. Elliott, H. Hengartner, and R. M. Zinkernagel. 1998. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J. Exp. Med. 187:1383.*
28. Battegay, M., S. Cooper, A. Althage, J. Baenzinger, H. Hengartner, and R. M. Zinkernagel. 1991. Quantification of lymphocytic choriomeningitis virus with an immunological focus assay in 24 or 96 well plates. *J. Virol. Methods 33:191.*
29. Smith, A., M. Wikstrom, and B. Fazekas de St. Groth. 2000. Visualizing T cell competition for peptide/MHC complexes: a specific mechanism to minimize the effect of precursor frequency. *Immunity 13:783.*
30. Laouar, Y., and I. N. Crispe. 2000. Functional flexibility in T cells: independent regulation of $CD4^+$ T cell proliferation and effector function in vivo. *Immunity 13:291.*
31. Butz, E., and M. Bevan. 1998. Massive expansion of antigen-specific $CD8^+$ T cells during acute virus infection. *Immunity 8:167.*